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Sepsis and Inflammatory Response Mechanisms: An activity stress model in humans

Roy J. Shephard, MD, PhD

Faculty of Physical Education & Health
Dept. of Public Health Sciences
University of Toronto, Toronto ON M5S 2W6

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Defence and Civil Institute of Environmental Medicine
1133 Sheppard Avenue West
Toronto, ON M3M 3B9

Scientific Authority
Pang Shek
(416) 635-2127

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Executive Summary

Military operations and training programs may be very rigorous, involving not only prolonged periods of heavy physical activity, but also exposure to extreme environments, such as hot or cold temperatures. The effects of such challenges on a soldier's health are complex, in part because of interactions between the various stressors. It is known that military personnel participating in lengthy and physically challenging operations and training are predisposed to a decrement in immune function and an increased susceptibility to infectious disease. From an occupational health perspective, immune suppression could impair both physical and mental performance. This study examined interactions of the exercise-induced immunomodulation with various forms of environmental stress, explores mechanisms, and examines implications for the overall health of military personnel.

The experimental design was targeted at elucidating the basic process of exercise-triggered inflammation, including issues of lymphocyte recirculation and activation. It further examined the immune disturbances induced by acute and chronic exercise. Possible exacerbation of exercise-induced changes by adverse environments, particularly heat and cold exposure was also investigated. Our study revealed that all key distinguishing features of a classical inflammatory response are detectable in an exercising individual, namely mobilization and activation of granulocytes, lymphocytes, and monocytes; release of inflammatory factors and soluble mediators; involvement of active phase reactants; and activation of reactive humoral cascade systems. In essence, exercise was found to trigger a sequence of inflammatory reactions remarkably similar to that of other inciting events, such as trauma, infection, and clinical stress.

We conclude that strenuous exercise can trigger the inflammatory cascade, and physical exercise and training represent an acceptable, and possibly a good model for the study of subclinical inflammatory responses in humans. Much further research is needed to assess the extent of these immune disturbances under various circumstances. There is as yet little definitive information as to how these changes may modify clinical resistance to disease. However, a variety of counter-measures are already available. These include task modification, the use of protective clothing, psychotherapy, nutritional and pharmacological aids, and an appropriate selection, acclimation and training of personnel. It may be necessary to exploit all of these approaches to optimize the operational efficiency of military personnel in extreme environments.

Sommaire

L'entraînement et les opérations militaires peuvent être physiquement très exigeants, non seulement parce qu'il y a de longues périodes d'activité physique intense, mais aussi parce qu'on peut être exposé à des conditions extrêmes comme une chaleur ou un froid intenses. Les effets que de telles exigences peuvent avoir sur la santé des soldats sont complexes, en partie parce que les divers facteurs de stress en jeu interagissent. Il est connu que les militaires qui participent à des opérations ou à un entraînement de longue durée physiquement exigeants ont une prédisposition à l'affaiblissement immunitaire et une sensibilité accrue aux maladies infectieuses. Dans le domaine de l'hygiène professionnelle on sait que l'immunosuppression peut nuire à la performance, tant physique que mentale. Dans l'étude présentée ici, nous examinons les interactions de l'immunomodulation résultant de l'exercice en interaction avec divers facteurs de stress environnementaux ainsi que les mécanismes en jeu, et nous regardons quelles en sont les conséquences pour la santé générale des militaires.

L'expérience que nous avons préparée devait nous permettre de comprendre le processus de base de l'inflammation déclenchée par l'exercice, y compris les aspects liés à la recirculation et à l'activation des lymphocytes. Nous avons aussi examiné les perturbations immunitaires résultant de l'exercice aigu et chronique, ainsi que l'aggravation possible des changements causés par l'exercice sous l'action de facteurs environnementaux nuisibles, plus particulièrement l'exposition à une chaleur ou à un froid extrêmes. Nos travaux ont révélé que chez un sujet faisant de l'exercice, on retrouve les principales caractéristiques de la réaction inflammatoire classique, soit la mobilisation et l'activation des granulocytes, des lymphocytes et des monocytes; la libération de facteurs inflammatoires et de médiateurs chimiques solubles, l'intervention de réactifs en phase active et l'activation des cascades de la réaction humorale. Essentiellement, nous avons constaté que l'exercice déclenche une série de réactions inflammatoires remarquablement semblables à celle que mettent en branle d'autres événements déclencheurs comme les traumatismes, les infections et le stress clinique.

Nous sommes parvenus à la conclusion que l'exercice intense peut déclencher une cascade inflammatoire, et que l'exercice et l'entraînement représentent un modèle

acceptable, voire adéquat, pour l'étude des réactions inflammatoires sub-cliniques chez l'humain. Il reste beaucoup de travail à faire pour déterminer l'importance de ces perturbations immunitaires dans diverses circonstances. Pour l'instant, peu de données concluantes nous renseignent sur la façon dont ces changements peuvent modifier la résistance clinique aux maladies. Toutefois il existe déjà toute une variété de mesures de lutte, notamment la modification des tâches, l'utilisation de vêtements protecteurs, la psychothérapie, les moyens nutritionnels et pharmacologiques et la sélection, l'acclimatation et l'entraînement adéquats des militaires. Il pourrait être nécessaire d'exploiter toutes ces approches pour optimiser l'efficacité opérationnelle des militaires en conditions extrêmes.

Abstract

Sepsis is a major cause of morbidity and mortality in combat casualties. A major problem in developing successful treatment has been the lack of appropriate human experimental models. Conclusions from animal experimentation have been suspect because of inter-species differences in the nature and time course of inflammatory reactions from those encountered in human surgery. Prolonged and strenuous physical activity can in itself cause substantial clinical injury, potentially causing an excessive inflammatory reaction and immunosuppression which mirrors that seen following surgical trauma.. This has opened up prospects of developing a technique that would permit controlled studies of adverse immune reactions to trauma. The objectives of this contract were thus to develop an exercise model that maximized cellular and humoral immune changes, and to use this model to explore patterns of secretion of pro- and anti-inflammatory cytokines and hormones during the stress of heavy exercise.

A laboratory comparison of brief, near maximal effort, sustained aerobic exercise, and a circuit of resistance exercise found that, contrary to expectations, sustained aerobic exercise above the anaerobic threshold yielded the reactions most typical of trauma. However, no mode of laboratory exercise induced a sustained and prolonged inflammatory response. In contrast, six hours of competitive cycling induced large increases in the secretion of pro-inflammatory cytokines. The depression of immune function induced by many forms of laboratory exercise seems too brief to have great practical importance for health. If such changes were induced several times per week, as in a sustained operation, there might be a cumulative adverse effect on immuno-surveillance and health experience. However, field studies of a basic training course at CFB Meaford found no adverse effects on health.

Given that laboratory exercise has only moderate effects on immune function, trials of counter-measures for sepsis will require either tests during more intensive training, such as the US Ranger training, or an exacerbation of the immediate inflammatory response by exposure to the combined stressors likely in combat, for example, extremes of heat and/or cold, and a negative energy balance. There seems some increase in resting immune function as individuals become trained, and this can partially offset the adverse effects of the inflammatory reaction.

Future research on exercise models of sepsis should concentrate upon the development of more marked immune changes through combinations of stressors, the testing of additional pro- and anti-inflammatory cytokine responses as concentrations of these substances fall within detection limits, and an examination of the protective effects of other anti-oxidants and nutritional supplements.

Résumé

Les infections sont l'une des principales causes de morbidité et de mortalité chez les blessés de guerre. Jusqu'ici, il a été difficile de mettre au point un traitement efficace, notamment parce qu'il n'existait pas de modèle expérimental humain adéquat. Les constatations découlant de l'expérimentation animale sont jugées suspectes parce que la nature et l'évolution des réactions inflammatoires diffèrent d'une espèce à l'autre et de ce qu'on observe en chirurgie humaine. L'activité physique prolongée et intensive peut entraîner des dommages cliniques assez importants et déclencher une réaction inflammatoire excessive ainsi qu'un effet d'immunosuppression correspondant à ce qu'on voit après une chirurgie. Avec l'observation de ce phénomène est apparue la possibilité de mettre une technique au point pour étudier en conditions contrôlées les réactions immunitaires nuisibles dues à des traumatismes. Les objectifs du projet décrit ici étaient donc de mettre au point un modèle d'exercice permettant de changer au maximum les fonctions immunitaires cellulaires et humorales, et d'utiliser ce modèle pour étudier la sécrétion des hormones et des cytokines pro-inflammatoires et anti-inflammatoires durant le stress d'un exercice intense.

En comparant au laboratoire les effets d'un bref exercice, exécuté en aérobie constante près du niveau d'effort maximal, à ceux d'une série d'exercices de résistance, on a constaté que, contrairement à toute attente, l'exercice en aérobie constante au-dessus du seuil d'anaérobiose entraîne des réactions très caractéristiques d'un traumatisme. Toutefois, aucune forme d'exercice en laboratoire n'a déclenché de réaction inflammatoire constante et durable. Par contre, six heures de vélo de compétition a fait augmenter dans une mesure importante la sécrétion de cytokines pro-inflammatoires. Il semble que la dépression de la fonction immunitaire que de nombreuses formes d'exercice de laboratoire ont entraînée ne dure pas assez longtemps pour avoir une véritable incidence sur la santé. Si ce genre de changement survenait plusieurs fois par semaine, comme il arrive durant une opération militaire de longue durée, il pourrait donner lieu à un effet nuisible cumulatif pour l'immuno-surveillance et l'expérience de santé. Toutefois, lorsque l'entraînement de base a été examiné dans des études réalisées

sur le terrain à la base des Forces canadiennes de Meaford, rien ne dénotait d'effet nuisible pour la santé.

Comme l'exercice en laboratoire n'a que des effets modérés sur la fonction immunitaire, pour faire l'essai de moyens de lutte contre les infections, il faudra des exercices plus intensifs, comme ceux de l'entraînement des US Ranger, ou une exacerbation de la réaction inflammatoire immédiate par exposition à l'ensemble des facteurs de stress susceptibles d'intervenir au combat, comme, par exemple, un froid et/ou une chaleur extrêmes et un bilan énergétique négatif. Par ailleurs, la fonction immunitaire de repos semble s'accroître à mesure que l'entraînement progresse, ce qui peut contrebalancer en partie les effets nuisibles de la réaction inflammatoire.

Il conviendrait dorénavant que la recherche sur les modèles d'exercice pour l'étude des infections soit axée sur l'obtention de changements immunitaires plus marqués par la combinaison de facteurs de stress, l'évaluation d'autres réactions pro-inflammatoires et anti-inflammatoires faisant intervenir des cytokines lorsque leur concentration est comprise entre les limites de détection et l'étude des effets protecteurs d'autres anti-oxydants et suppléments nutritifs.

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1. Introduction

Sepsis constitutes a major cause of morbidity and mortality in combat casualties with severe wounds. Uncontrolled sepsis probably plays a central role in the development of multiple organ failure. Improvements in resuscitation, anaesthesia, and critical care medicine have made important contributions to survival following major surgery and trauma. Despite these medical and surgical advances, service patients remain at high risk of morbidity and mortality from postoperative or post-traumatic sepsis. The major problem in implementing a successful treatment modality stems from the difficulty in controlling or reversing the exaggerated systemic inflammatory response triggered by microbial products released into the circulation, which stimulates the release of a complex series of cytokines and mediators. Progress in evolving treatments that could minimize the sequelae of post-trauma inflammatory complications have been hampered by the lack of appropriate human experimental models.

Conclusions drawn from animal experimentation have been suspect because of inter-species differences in gross and cellular physiology of the immune system, and differences in the time course of inflammatory reactions from those encountered in human surgery. The one human model examined to date has been based on the injection of small amounts of endotoxin. However, the doses of endotoxin used in such models represent only a small fraction of those encountered in the complications of trauma, and reactions to this stimulus have differed in some respects from those encountered in sepsis.

Recent research has suggested that prolonged and strenuous physical activity can in itself cause substantial clinical injury, with the potential for an excessive inflammatory reaction and immuno-suppression. Under a separate research contract, the contractor organized an international symposium at DCIEM to explore this question further. A panel of some 20 experts presented papers on this topic, and the proceedings were collected, edited, and published as a special issue of the Canadian Journal of Physiology and Pharmacology (76: 469-597, 1998). Detailed reviews of this concept were also published with support of the present contract (see Appendix One), and a fuller exploration of immune responses to vigorous physical activity and training in a variety of normal and stressful environments were discussed in a major monograph (1).

2. Trauma and Stressful Physical Activity

Our research approach has opened up new prospects for controlled studies that can explore treatment options for service patients who show adverse immune reactions to trauma. Detailed studies in our own laboratories and elsewhere have indicated some striking parallels between the

complex regulatory and counter-regulatory responses to surgical trauma and the reactions to either a single bout of exhausting exercise, or a prolonged and systematic period of heavy training. The changes in immune response induced by a prolonged bout of intensive exercise are not only readily measured, but are of sufficient clinical importance as to provide an "open-window" that allows infection by a number of opportunistic micro-organisms. Infection is a further factor that can have adverse impact on military preparedness; this aspect of the contract has been the subject of field research during basic infantry training, as described in Military Medicine (2), and has led to several in-depth reviews, including other publications in Military Medicine (3) and the Physician and Sportsmedicine (4).

Specific changes that have been observed following both surgical trauma and stressful physical activity include:

1. Cellular infiltration of the active tissue by neutrophils and macrophages, the extent of infiltration being correlated with the extent of sub-clinical damage to the muscle.
2. Activation of both neutrophils and macrophages, as shown by increased plasma levels of myeloperoxidase, elastase and neopterin.
3. A local release of eicosanoids and the monokines IL-1, IL-6 and TNF-α, with systemic overflow and tissue damage apparently related to the extent of IL-1 and IL-6 release and their persistence in the injured tissue.
4. A modulation of the in vitro production of cytokines by peripheral blood mononuclear cells (e.g. a late decrease in the production of IL-1, IL-2, TNF- α and IFN–γ by mitogen-stimulated peripheral blood mononuclear cells, due in part to a down-regulation and in part to a shift in the $CD4^+/CD8^+$ ratio.
5. Suppressed NK cell counts and NK cell activity for up to 7 days following severe exercise.
6. Suppressed in vitro production of immunoglobulins, with reduced immunoglobulin concentrations in serum and nasal washings, and

33. Suppressed proliferation of T cells in response to mitogens.

The initiation of an excessive inflammatory reaction appears to depend upon an up-regulation of T-helper 1 (Th1) cells, modulated by IL-8, and the key to restoration of a more appropriate balance of Th1 versus Th2 activity is a down-regulation of the Th1 cells, modulated by IL-10 and TGF-β. Unfortunately, these cytokines and the corresponding mRNAs only "overflow" into the blood stream in minuscule quantities. There is thus a need to maximize the experimental injury reaction

(within ethical limits) to allow a study of its components and how they may be prevented or controlled by therapeutic agents. The present investigation has defined the optimal pattern of execise for eliciting a change in immune function, has examined the mechanisms for this, and looked at potential modulation of responses by antagonists of endorphin and prostaglandin.

3. Research Scope

The broad plan of investigation, as contained in the initial work statements, has been reviewed, adapted in the light of experimental findings on the basis of regular monthly meetings with the Technical Authority. The contract has provided scope for the training of 6 post-doctoral fellows, and 5 graduate (3 doctoral and 2 masters) students. Opportunity has also been taken to conduct research in the field, in collaboration with CFB Meaford, the Environmental Physiology Laboratories of the US Army Research Institute of Enivronmental Medicine, and the French Armed Services.

With appropriate acknowledgment of contract support, an exceptional number of papers have been published and lectures presented in many parts of the world (to date, 57 peer-reviewed articles have been published or accepted for publication, and several others are at various stages in preparation, along with 26 abstracts, and 34 presentations), as listed in Appendix Three.

The initial objective was to develop an exercise model that maximized change in terms of disturbance of NK cell count and activity, CD4+/CD8+ ratio, and the production of key cytokines. This model was then used to study alterations in pro- and anti-inflammatory hormones, plus PGE2 and cortisol levels during exercise. Because of difficulties in identifying the small quantities of cytokines present in the plasma, measurements of the corresponding mRNAs and intracellular cytokine concentrations were examined in peripheral blood mononuclear cells. Field testing of the impact of a rigorous basic training course on immune function was conducted at CFB Meaford. Laboratory trials demonstrated the plasma accumulation of prostaglandin, and the reversal of certain exercise-induced changes by a non-steroidal antiinflammatory agent (naloxone).

Further research demonstrated exacerbation of the initial inflammatory response by imposition of combined stressors likely in combat or rigorous training (for example, extremes of heat and/or cold), and the testing of potential remedies (inhibitors of prostaglandins and beta-endorphin), along with more fundamental work on the pathways of leukocyte recirculation during severe stress, and the intracellular sources of pro- and anti-inflammatory cytokines.

4. Optimization of Model of Inflammatory Response

4.1 Overall pattern of physical activity and health.

Detailed reviews have examined the interactions between intensity, frequency, duration and volume of physical activity and overall health, both acute and chronic, including susceptibility to infections and cancer. The impact of the various patterns of activity on NK cell counts and function have been the subject of a formal meta-analysis.

The meta-analysis accumulated data from 94 studies describing the NK cell response of some 900 subjects to acute and chronic (5-6). NK cell numbers, indicated in terms of $CD3^-CD16^+CD56^+$, $CD16^+$ or $CD56^+$ phenotypes, and cytolytic activity were expressed per 10,000 peripheral blood mononuclear cells or in terms of lytic units. Acute exercise was categorized as sustained moderate (50-65% of aerobic power), sustained vigorous (>75% of aerobic power), brief maximal or "supramaximal", prolonged, eccentric or resistance, or repeated exercise. In general, there was a marked increase of NK cell count at the end of exercise, probably attributable to a catecholamine-mediated demargination of cells. Following exercise, cell counts dropped to less than a half of normal for a couple of hours, but except in unusual circumstances, normal resting values were restored within 24 hours. If activity was both prolonged and vigorous, the decrease in NK cell counts and cytolytic activity began during the exercise session. Although the usual depression of NK cell count seems too brief to have great practical importance for health, if such changes are induced several times per week, as in a sustained operation, there could be a cumulative adverse effect on immuno-surveillance and health experience. There is a weak suggestion of an offsetting increase in resting NK cell counts and cytolytic action as individuals become trained, and this merits further exploration under conditions where the effects of recent training sessions can be carefully controlled.

4.2 The effect of training volume

The impact of training five rather than three times per week was studied in a simple cross-sectional comparison, with results suggesting that whereas the enhancement of aerobic power was greater with the five days/week stimulus, a negative energy balance appeared to suppress immune function.

Responses to 60 minutes of training three times (9 subjects) or five times (18 subjects) per week at 70-85% of maximal heart rate were compared to control data on 6 subjects (7-8). The lighter volume of training increased aerobic power by only 8%, without loss of body mass, whereas the heavier training was associated with a negative energy balance (8.2% decrease of body mass, 18.1% loss of body fat). There was some evidence that immune changes were more beneficial with the lesser volume of training: increase of resting CD16+ count 27% vs 21%, post-exercise suppression

of NKCA averted more with three than with five sessions, decreased resting CD19+ count with 5 sessions per week.

4.3 The effect of exercise type

Immune responses to three forms of exercise stress: brief, near maximal effort, sustained aerobic exercise, and a circuit of resistance exercise have been compared in terms of cellular and humoral indices. The cellular and humoral evidence of inflammatory response showed a different ranking to muscle soreness and creatine kinase release, prolonged endurance exercise yielding the reactions most typical of the inflammatory response.

It was hypothesized that (a) muscle injury would be greater with the resistance circuit than with the all-out or prolonged exercise, and (b) immune changes might provide an indication that such exercise had induced an inflammatory response, supplementing the information provided by traditional markers of exercise-induced muscle injury such as creatine kinase (CK) or delayed-onset muscle soreness (DOMS). Eight healthy males (mean age 24.9 $\pm$ 2.3 yr), $\dot{V}O_{2max}$ = 43.0 $\pm$ 3.1 $ml.kg^{-1}.min^{-1}$), were each assigned to a sequence of four experimental conditions, using a randomized-block design. Subjects performed 6 min of all-out cycle ergometer exercise at 90-97% of $\dot{V}O_{2max}$, 2 h of cycle ergometer exercise at 60-65% of $\dot{V}O_{2max}$, a standard circuit training routine consisting of biceps curl, knee extension, hamstring curl, bench press and leg press (3 sets of 10 repetitions at 60-70% of 1RM for each station, with 1 min rest between sets, and 3 min rest between stations), or remained seated for 5 h.

Blood samples were analyzed for total leukocytes and subpopulations, total T cells ($CD3^+$), T helper/inducer cells ($CD3^+CD4^+$), T suppressor/cytotoxic cells ($CD3^+CD8^{bright+}$), B cells ($CD19^+$), cytolytic T cells ($CD3^+CD16^+/56^+$), and natural killer cells ($CD3^-CD16^+/56^+$). All-out and prolonged exercise induced similar changes in cell counts, both of which were generally larger than those induced by the circuit-training exercise. However, all-out exercise and circuit training both resulted in a significant and longer lasting decrease of the $CD4^+/CD8^+$ ratio than did the prolonged exercise bout. The immediate ranking of cellular responses (all-out similar to prolonged, >eccentric exercise) did not match that of symptoms and CK release 1-3 days post-exercise (eccentric>prolonged>all-out exercise), and contrary to expectation responses were most marked with anaerobic and prolonged aerobic exercise, rather than with the eccentric form of exercise (10-11).

All three types of exercise induced a significant rise in circulating NK cell counts, greatest for all-out exercise, slightly less for prolonged exercise, and weakest with circuit training; baseline values

were regained 3 h post-exercise. Total cytolytic activity increased significantly with all-out and prolonged exercise, with a parallel but non-significant trend in response to circuit training. Plasma IL-6 tended to rise after all-out and circuit training, and showed a significant increase, peaking at sixfold 3 h post-prolonged exercise. TNF-α showed a small increase with prolonged exercise only, peaking at less than twofold 72 h post-exercise. IL-10 was significantly reduced by all-out exercise, but unchanged with the other two forms of activity. Evidence of muscle injury was seen with soreness in the chest, arms or legs 24-48h after circuit training (7 of 8 subjects), and the legs (2 of 8 subjects) following prolonged endurance exercise. Creatine kinase levels tended to rise 24 h after circuit training and prolonged exercise. Signs of muscle injury showed a different ranking from the plasma cytokine data, possibly because the exercise-induced inflammatory response is modified by humoral and cardiovascular correlates of exercise. Prolonged endurance exercise yielded the profile most typical of an inflammatory (9-11).

Although all three types of exercise approached the tolerance limit of sedentary subjects, none were of sufficient severity to induce a substantial and persistent inflammatory response. Future research on this topic could possibly utilize athletic samples who might be more prepared to exercise to the point of causing muscle micro-trauma.

5. Cytokine and Humoral Responses

5.1 Impact of prolonged exercise on cytokine release and leukocyte circulation

As a model of an extreme bout of endurance exercise, observations were made on a small sample of competitive cyclists 10-25 min and 150 min after completion of a 6 h 250 km road race in warm weather (12). Resting blood samples were collected from 6 amateur cyclists, 24 h before and at 10-25 min and 150 min after completion of a 250 km road race, completed in 404 ± 3.5 min. Heart rates over the race averaged 158 ± 17 beats/min, 81% of peak heart rates for the sample. There were increases in total leukocyte (100%), granulocyte (84%), lymphocyte (11-15%) and monocyte (4-5%) counts, and decreased $CD3^-CD16^+CD56^+$ (11%) counts for at least 2.5 h post-exercise. Cell counts of the $CD3^+CD8bright^+$ cytotoxic lymphocytes were depressed by 50% 150 min following competition. A significant increase in $CD4^+$ cells expressing the IL-2R α chain was evident 150 min after competition, suggesting either an activation of these cells, or a preferential clearance of naive cells from the circulation. IL-6 concentrations were greatly increased, both at 10-25 min (45-fold) and at 150 min (25-fold) after competition. Resting TNF-α concentrations were approximately doubled at both time points after competition, as would be anticipated from the increases in IL-6. Plasma levels of IFN-γ, IL-10 and IL-12 were below the detection thresholds of

the available kits at all time points. These results suggest that the 6.5 hour race induced a pro-inflammatory response.

5.2 Response of leukocytes and cytokines to moderate aerobic training

Comparisons before and after 12 weeks of moderate aerobic training in 9 previously sedentary males show that $CD16^+CD56^+$ NK cell numbers are elevated in trained individuals, and that acute, exercise-induced fluctuations in lymphocyte subsets ($CD4^+$, $CD8^+$ and $CD19^+$), activation markers ($CD25^+$ and CD 122^+), lymphocyte proliferation and IL-2 release are blunted after training, all of these various changes suggesting that moderate endurance training may modulate host defence and the extent of exercise-induced inflammatory responses.

5.3 Impact of anti-inflammatory agents and endorphin antagonists

Some authors have suggested that β-endorphin is responsible for the exercise-induced changes in circulating NK cell count and activity; however, if the cause is an inflammatory response, there is likely a release of prostaglandin which can be countered by non-steroidal anti-inflammatory agents. A double-blind randomized block trial used oral naloxone to counter β-endorphin, and indomethacin to counter prostaglandin release. The β-endorphin antagonist had no effect other than to reduce the exercise-induced increment of β-endorphin, but prostaglandin levels did increase during activity, and the prostaglandin antagonist was able to reverse the suppression of NK cell activity post-exercise, in keeping with the inflammatory hypothesis.

A double-blind trial examined the influence of oral prostaglandin inhibitor (indomethacin, 75 mg/d for 5 days) on exercise-induced changes in NK cell counts and NKCA in 10 young men who performed 2 h of cycle ergometer exercise at 65% of aerobic power (14). During exercise, NK cell counts and activity showed the anticipated increase in both experimental and control conditions. In the placebo experiments, total NKCA was suppressed by 28% 2 h after exercise, and K-562 tumor cell lysis was negatively correlated with the post-exercise increase in PGE_2 (which averaged 36%). NK counts were unchanged post-exercise, but $CD45^+$ counts were increased. After indomethacin treatment, the post-exercise increase in PGE_2 was eliminated, and the suppression of NKCA was completely reversed. Thus, the post-exercise reduction in NKCA seems due to changes in circulating PGE_2 rather than a differential lymphocyte redistribution.

The impact on immune function of a single 50 mg dose of the opioid antagonist naltrexone hydrochloride, ingested 60 min before 2 h of cycle ergometry at 65% of aerobic power was tested in the same sequence of double blind trials on 10 healthy young men (15-16). Placebo and experimental trials showed statistically significant elevations of β-endorphin at 90 and 120 min of

exercise, with recovery of normal resting values 120 min post-exercise, changes being significantly attenuated by the naltrexone. NK cell counts and NK activity were increased throughout exercise, but again no difference was seen between placebo and naloxone trials. Moreover, naltrexone did not alter the exercise-induced change in cell adhesion molecule expression (CD2 and CD11a), nor did it modify the exercise-induced changes in growth hormone and total cortisol concentrations. Changes in NKCA reflected mainly the changes in NK cell count.

6. Impact of Trafficking between Circulating and Non-circulating Leukocytes

The leukocytes observed in the peripheral blood account for only 1-2% of the total number in the body. It is thus important to check how far the observed exercise-induced changes in cell characteristics such as cytolytic activity reflect an alteration in the expression of adhesion molecules and trafficking of cells between the circulation and tissue depots. A study of $CD4^+$ cells demonstrated a selective mobilization of cells having the $CD45RO^+$ memory phenotype; however, the mobilization of $CD8^+$ cells was distributed much more uniformly between naive and memory cells (18). The cells that are mobilized carry high levels of the adhesion marker CD11a, and mobilization of cells seems proportional to initial densities of this molecule.

Under normal resting conditions, naive and memory T cells follow distinct pathways of recirculation. During periods of heavy physical exercise, T cells with a high-density expression of the alpha chain of LFA-1 (ie $CD11a^{hi+}$) are preferentially mobilized to the blood (19). Since memory T cells display high levels of CD11a expression and are the predominant phenotype within the marginal lymphocyte pool, it was anticipated that the majority of T cells mobilized to the blood during heavy physical exercise would be of memory phenotype. This was tested in 12 young males before, during and after 40 min of cycle ergometer exercise at 65% of aerobic power. $CD4^+$ cells increased 23% in response to exercise, with 80% of these cells having a memory ($CD45\ RO^+$) phenotype. However, a 44% increase in $CD8^{hi+}$ cells was attributable to much closer proportions of naive (43%) and memory (57%) cells. Thus, the mobilization of $CD4^+$ cells during exercise is linked in some way to memory phenotype.

Catecholamines are thought to reduce adhesion forces, changing the surface density or avidity state of specific receptors, and thus promoting the detachment of previously adherent cells. Studies of 10 males who exercised for 2 h at 65% of aerobic power showed increases in leukocyte counts [$CD56^+$ (330%), $CD8^{hi+}$ (105%) and $CD4^+$ (30%)]. The majority of lymphocytes mobilized expressed high levels of CD11a, changes in subset concentrations being positively correlated with initial resting levels of CD11a ($CD56^+$ 270 MFU; $CD8^{hi+}$ 176 MFU; $CD4^+$ 107 MFU) (20-21).

The mobilization of total leukocytes, granulocytes, monocytes and lymphocytes during the early stages of 2 h cycle ergometry at 65% of aerobic power is unaffected by naloxone administration, but there are later minor changes, including a greater granulocyte number at 2 h of exercise and a reduction in lymphocyte count 2 h post-exercise.

7. Pathways of Cell Mobilization

An understanding of the pathways of cell mobilization is critical to efforts at modulating the immune response to heavy exercise and a stressful environment. The contribution of the lymphatic system to the lymphocytosis that is seen after the simulation of stress exposure by infusion of epinephrine has remained controversial. Using sheep we investigated the effect of a physiological dose (1 mg) of epinephrinei on lymph flow, cell concentration and lymphocyte subsets in efferent subcutaneous lymph. Simultaneously, blood leukocyte numbers, differential and lymphocyte subsets were determined. Additionally, blood and lymph pools of lymphocytes were monitored. A significant acute increase in lymph flow was followed by a post-injection decrease in flow and cellular output. No changes in lymphocyte subsets or pools of lymphocytes were seen in either blood or lymph. The timing of elevated plasma and lymph concentrations of epinephrinei and norepinephrine corresponded with increased lymph flow. Epnephrine injection causds no change in lymphocyte subset distribution, leukocyte concentration or pools of lymphocytes. A decrease in lymph flow and cellularity was documented post-injection, so we conclude that lymphatic tissue has no role in the leukocytosis seen after epmnephriei injection. Lymphocyte retention by lymph nodes, however, may contribute to post-injection lymphopenia.

8. Anaerobic Threshold as a Marker of the Critical Intensity of Effort

Further evidence on the critical intensity of exercise for development of an inflammatory response was obtained in studies conducted above and below the anaerobic threshold (23-24). The response to 30 min of exercise at 80% and 120% of ventilatory threshold was compared in 7 healthy young men. At 120% of threshold, NK counts increased during exercise, but dropped below resting values 15, 30 and 60 min post-exercise; NKCA was also depressed 30 min post-exercise, but activity per cell remained unchanged. At 80% of threshold, counts increased during exercise, but did not drop significantly below rest post-exercise.

A similar experiment, with exercise bouts limited to 5 min showed a significant suppression of NKCA 30 min after the 120% effort. Thus, even brief anaerobic effort seems sufficient to inhibit NKCA. In this study, PGE_2 did not change either during or post-exercise, but there were decreases in serum cortisol 15, 30 and 60 min post-exercise.

9. mRNAs and Intracellular Cytokines

Because of the difficulty in detecting many of the pro- and anti-inflammatory cytokines in plasma, the concentrations of mRNAs have been evaluated in peripheral blood mononuclear cells during and following exercise, and preliminary observations have also been made on intracellular cytokine concentrations (25). Despite demonstrable increases in plasma concentrations of IL-6 following exercise, mRNA concentrations and intracellular concentrations have appeared to remain unchanged in the circulating leukocytes. This suggests either an alternative source of cytokines (for example, macrophages within the muscles or myocytes), or the release of previously secreted cytokines. The preliminary data showing unchanged intracellular concentrations is rather against the latter hypothesis.

Ten healthy, recreationally active but otherwise untrained males ($\dot{V}O_{2max}$ = 48.8 ± 6.5 ml/[kg.min]) performed a 3 hour bout of physical activity at 60-65% of $\dot{V}O_{2max}$ (26). The activity comprised 60 min of cycle ergometry, 60 min of walking up an inclined treadmill (+12°), a further 60 min of cycle ergometry and a final period of two hours seated rest. On control days, randomly assigned, the subjects rested for 5 hours, at the same period of the day. Circulating levels of total leukocytes, granulocytes, monocytes and lymphocytes showed the expected increments during exercise and for at least two hours of recovery, but resting control values had been regained within 24 hours. Plasma concentrations of IL-6 increased about ten-fold, to 21.8 pg/ml, at the end of exercise, but this rise was transient. Plasma TNF-α rose almost fourfold, to 1.92 pg/ml, remaining elevated 2 and 24 hours post-exercise. IL-1-β was detectable only at the initial sampling point in resting subjects, but with exercise it became detectable before (0.19 pg/ml) and at the immediate end (0.59 pg/ml) but not after exercise. The accumulation of poly-adenylated mRNA was evaluated in peripheral blood mononuclear cells, using a competitive reverse transcription polymerase chain reaction. Messenger RNA levels of IL1- β, IL-6, and TNF-α did not change significantly from initial levels in either the exercise or the control condition. Thus gene expression, represented by IL1- β, IL-6 and TNF- α mRNA in circulating immunocytes does not seem able to account for the substantial plasma accumulation of certain cytokines during and immediately following exercise. Although exercise can be a potent modulator of the immune system, it does not stimulate peripheral blood mononuclear cells in the manner that is observed in infection, sepsis and massive trauma.

10. Field Trials

Opportunity was taken to study susceptibility to infections, leukocyte subset counts, cell proliferation, salivary immunoglobulin levels and the overall intensity of immune responses as assessed by skin reactions to seven common antigens as recruits participated in an 18.5 week basic

infantry training course at CFB Meaford (2). In view of earlier reports of adverse reactions to US Ranger and Cadet courses, we had anticipated that some immunosuppression might develop, increasing the susceptibility of participants to upper respiratory infections; if such a change had developed, we were planning to assess the protective value of anti-oxidant medication, administered in double-blind fashion. In fact, we were able to affirm that the course had no adverse effect on health, and field trials of anti-inflammatory agents will require either a more intensive training programme, or exposure to a combination of heavy physical activity and other types of stressor such as sleep deprivation, hyperbaric conditions, extremes of heat or cold.

The effect of an 18.5 week basic infantry training on innate and adaptive immunity (including lymphocyte proliferation, natural killer [NK] cell activity, *in-vivo* cell-mediated immunity and secretory IgA levels) was studied in 23 military personnel (mean ± SE, aged 22.0 ± 0.5 y). Aerobic power, body composition, and immune function were measured in subjects at the beginning and end of the course. All subjects self-reported symptoms of sickness in health logs using a pre-coded checklist. Data from this study indicate that subjects became leaner and maintained but did not increase their aerobic fitness by the end of the course. PHA-stimulated lymphocyte proliferation and NK cell activity were significantly enhanced over the period of the course. *In-vivo* cell-mediated immunity was assessed by a delayed-type hypersensitivity test (the Cell Mediated Immunity [CMI] - Multitest [Connaught Laboratories, North York, ON]) which tests cutaneous reactions to two toxoids [tetanus and diphtheria], three bacterial antigens [streptococcus, tuberculin, and proteus] and two fungal antigens [candida and trichophyton]) and a negative control (glycerin). The number of positive reactions and the extent of induration remained unchanged throughout the trial. Levels of secretory IgA as seen in specimens of saliva were lower by the end of the course, possibly because many of the recruits had ceased cigarette smoking. The incidence of infection remained stable throughout the course. These results indicate that the current pattern of basic infantry training does not have an adverse impact on the health status of recruits. Trials of anti-inflammatory medication would require either a more intensive training course, or exposure to a combination of prolonged endurance exercise and some form of environmental stress such as extremes of heat, cold, hyperbaric conditions, or sleep deprivation

11. Suggestions for Future Research

Further research should concentrate upon the development of more marked immune changes through combinations of stressors, the testing of additional cytokine responses, and an examination of the protective effects of various anti-oxidants and nutritional supplements (27).

Given the constraint that measurements of human cellular and cytokine responses to stress must be examined in specimens of peripheral blood rather than at the site of tissue injury, the concentrations

of many of the components of the immune response are below the detection threshold of currently available biochemical and immunological kits, whether measurements are made in the plasma, intracellularly or as mRNA. More marked changes should result from exposure to combinations of exercise with other stressors such as disturbances of sleep, extremes of heat and cold, and hyperbaric conditions. Accordingly, responses of key cellular elements, inflammatory and anti-inflammatory cytokines should be studied under the worst conceivable conditions of combat, e.g. combinations of sleep deprivation, heat and cold exposure. The effects of a number of these stressors on immune function independent of exercise have already been the object of detailed review during the course of this contract (28-31).

Assuming, as anticipated, that the combined stressors are more effective in inducing alterations in immune response, investigators should undertake detailed study of alterations in balance between Th_1 products (IL-2, IFNγ) and Th_2 products (IL-4, IL-5, IL-6, IL-10), as well as IL-8, TNF-α, TGF-β and leukotriene B4 levels, plus PGE_2 and cortisol levels.

Based on a fuller understanding of the inflammatory mechanisms determined from the established exercise stress models, the efficacy of appropriate prophylactic and/or therapeutic agents such as free radical antagonists, nutritional supplements and anti-inflammatory agents should be assessed in terms of reversal of cellular and humoral changes and modulation of responses to cutaneously administered antigens. Review of a number of possible therapeutic measures, including nutritional and vitamin supplements, has already been completed (32-33).

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Appendix 1
Immune Responses to Inflammation and Trauma

This review looks at parallels between exercise and septic reactions to trauma, examines interactions of the exercise-induiced immuno-modulation with various forms of environmental stress, explores mechanisms, considers possible antidotes, and examines implications for the overall health of military personnel.

Immune Responses to Inflammation and Trauma: A Physical Training Model

In addition to obvious military applications, the topics of inflammation and sepsis have growing clinical importance in an era where physicians must use antibiotics of declining efficacy in populations whose immune function has been compromised by extreme age or HIV infection. Whether the precipitating condition be severe trauma or extensive burns, the patient faces very dangerous complications- an excessive inflammatory reaction and a temporary stimulation of immune response, followed by severe immuno-suppression, shock, septicaemia, pulmonary and other microvascular lesions, organ failure and death.

The normal inflammatory reaction

Tissue injury normally induces a rapid but complex sequence of immune reactions. Injured cells and aggregated platelets produce large amounts of platelet activating factor (PAF), various growth factors, and IL-8 and related cytokines (Bagglioni et al., 1994; Northoff et al., 1995; Venable et al., 1993). A massive influx of neutrophils is stimulated. The platelet derived growth factor (PDGF) suppresses the p53 gene that normally restricts cell growth (Antoniades et al., 1994). It also induces a JE gene in arterial smooth muscle which codes for macrophage attractants (Taubman et al., 1992).

Macrophages move progressively to the injured tissue over the next few days. Like the neutrophils, they exert a phagocytic function, but they also secrete many growth factors, PAF, leukotrienes, prostaglandins and cytokines, including IL-1, IL-6, IL-8, IL-10, IL-12, TNF-alpha and IFN-beta (Adams & Hamilton, 1984; Sun et al., 1990). Consequences include a stimulation of growth (IL-1, IL-6, TGF-beta), activation of lymphocytes (IL-1, IL-6, IL-12, TNF-alpha; Lowry, 1993), attraction and activation of neutrophils (IL-8; Baggiolini et al., 1994), induction of adhesion molecules such as ICAM and CD18 on leukocytes and endothelial cells (Smith et al., 1989), complement activation (Sun et al., 1991), and cell death (Yamamura et al., 1992).

The stimulated lymphocytes in turn produce a vast array of cytokines and growth factors. This allows an expansion and differentiation of T cells and B cells, and an activation of NK cells and

macrophages (Rhind et al., 1995). The Th_1 cell subset produces predominantly IL-4 and IL-5, and is responsible for immediate hypersensitivity reactions, while the Th_2 subset produces delayed reactions via IL-2, IFN-gamma and TNF-beta (Yamamura et al., 1992).

Control of the inflammatory response

A variety of processes normally combine to control inflammation, preventing an excessive immune response:

1. Heat shock proteins. Heat shock proteins (HSP) serve as free radical scavengers, and HSP70 counters the TNF-induced activation of phospholipase A2, a key enzyme in the inflammatory process (Jäättelä, 1993).

2. Anti-oxidant vitamins. The antioxidant vitamins (A, C, and E) play an important protective role (Amelink et al., 1991), although it is less certain whether there is any clinical advantage in attempting to augment normal body stores of these substances.

3. Acute phase reaction. The acute phase reaction (induced by IL-6 in the presence of adequate cortisol levels) reduces transferrin synthesis (thus reducing serum iron, an important catalyst of free radical formation), while increasing hepatic synthesis of complement, fibrinogen and haptoglobin (Cannon et al., 1990; Northoff & Berg, 1991).

However, C-reactive protein, also a part of the acute phase response, can cause inflammation (Yamada et al., 1990).

4. Receptor antagonists. Another important down-regulating mechanism involves the production of receptor antagonists such as IL-1ra which block the cytokine receptors (Lowry, 1993) and soluble receptors such as sIL-2r which inactivate free cytokines (Lahat et al., 1993).

5. Prostaglandins. The overall effect of arachidonic acid metabolites is a stimulation of inflammation, and many patients thus benefit from the administration of NSAIDS. However, prostaglandins of the E series down-regulate the production of cytokines by macrophages and lymphocytes (Rivkind et al., 1989; Takayama et al., 1994).

6. Cytokines. Although IL-1 and IL-6 have a direct stimulatory action, they also increase corticosteroid levels by an action on the hypothalamic pituitary axis (Northoff & Berg, 1991). Cortisol is a highly effective anti-inflammatory agent; it decreases cytokine production and cell proliferation, and it decreases the production of macrophage attractants by smooth muscle. Other cytokines (particularly IL-10 and TGF-beta) have strong immunosuppressive actions in their own right. IL-10 can down-regulate monocyte expression of HLA-DR; this leads in turn to a suppression of Th_1 lymphocytes (with a reduced production of IL-2 and IFN-gamma), suppression of NK and LAK activity, reduced T cell proliferation and reduced antibody production. IL-10 also upregulates IL-1ra, blocking the immune cascade; the resultant decrease in the output of IL-2 can sometimes lead to a disastrous overall suppression of immune function (Kühn et al., 1993).

Excessive inflammatory response

An excessive inflammatory response can damage tissues through the production of lysosomal enzymes, elastase and reactive oxygen species.

Stimuli to free radical production include IL-8, TNF-alpha, leukotriene B4, PAF, endotoxin, and activated complement compounds (Baggiolini et al., 1994; Venable et al., 1993; Ward et al., 1988). Free radical damage extends the original injury, and can set up a vicious cycle, with a progressively increasing activation and influx of leukocytes.

An over-production of TNF seems one likely cause of an excessive inflammatory response. In addition to direct cytotoxicity, it can activate coagulation and complement cascades, and leukocytes. PAF, arachidonic acid metabolites and IFN-gamma probably serve to exacerbate the inflammation.

Excessive counter-regulation

Reactions to control the inflammatory response can also be excessive. There is then a phase of immunosuppression, when the body shows an increased vulnerability to opportunistic infections (Brenner et al., 1994). However, it is still disputed how far the clinical problems of the Multiple Organ Distress Syndrome (MODS), the Systemic Inflammatory Response Syndrome (SIRS) and the Acute Respiratory Distress Syndrome (ARDS) are due to the initial excessive inflammation, and how far they are a later consequence of immuno-suppression or leakage into the circulation of gram negative organisms and endotoxin from the damaged gut (Jones et al., 1990; Moore et al., 1995). Some authors find a suppression of granulocyte chemotaxis and phagocytosis in patients affected by these conditions (Grogan & Miller, 1975; Maderazo et al., 1983), but others report overactivation of the granulocytes in patients with an adverse clinical course (Christou & Tellado, 1989; Mannick, 1993).

The monocytes may show a reduced expression of HLA-DR antigens, thus limiting their ability to activate T cells (Volk et al., 1990). An increased secretion of prostaglandins may further suppress the ability of Th_1 cells to secrete IL-2 and to mount a delayed hypersensitivity reaction (Horgan et al., 1988). Finally, in part because of the lesser availability of IL-2, NK cell activity is usually impaired by an excessive inflammatory reaction (Blazar et al., 1984; Meakins et al., 1977).

Experimental models of inflammation and sepsis

A greater understanding of the processes of inflammation and sepsis is an essential preliminary to the development of effective treatments that could minimize the dangerous sequelae of blunt trauma, injury, burns and blood loss. Progress in this area has been hampered by the lack of appropriate human experimental models.

Animal models of sepsis have allowed drastic experimental interventions, but conclusions have been difficult to apply to the regulation of inflammation in humans because of inter-species differences in gross and cellular pathophysiology.

Human investigations have endeavoured to trace meaningful correlations between the mortality from sepsis or its complications and the cellular or humoral immune responses observed at an earlier stage during the treatment of severe injuries and burns. The one experimental model as yet applied to humans examined reactions to the injection of small amounts of endotoxin (Santos & Wilmore, 1996). However, the doses used were but a small fraction of those encountered in the complications of trauma, and reactions to the endotoxin differed in some respects from those encountered in sepsis.

There is thus considerable interest in the possibility of using exercise and physical training as models of inflammation and sepsis. There is growing evidence that very strenuous physical activity can cause substantial sub-clinical injury, initiating an excessive inflammatory reaction and immunosuppression (Northoff et al., 1995) that in many respects mimics the immune reactions observed in clinical sepsis. Such observations open up the exciting prospect of controlled studies, where heavy exercise can be used to cause graded and well-defined amounts of muscle trauma, with the opportunity to explore mechanisms and test various potential methods of preventing and treating an excessive inflammatory response.

The present project reviews the basic processes of inflammation, including issues of lymphocyte recirculation and endotoxaemia, examines the immune disturbances induced by acute and chronic exercise and considers parallels between clinical sepsis and immune responses to exercise. It then considers factors modulating immune responses, including circulating levels of various cytokines, nutritional status, an altered expression of adhesion molecules, and the possible intervention of reactive species. A third section explores possible exacerbation of exercise-induced changes by adverse environments, particularly heat exposure, and interactions with sleep and wakefulness. The final reports focus on selected clinical applications: aging, infection and neoplasia.

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Appendix 2

Physical Exercise as a Human Model of Limited Inflammatory Response

An inflammatory response is the outcome of a fundamental series of humoral and cellular reaction cascades in response to infection, tissue injury, and related insults. An excessive response is commonly seen under the pathological conditions of trauma, sepsis and burns. Most, if not all of the distinguishing features of a classical inflammatory response are detectable in an exercising individual, namely mobilization and activation of granulocytes, lymphocytes, and monocytes; release of inflammatory factors and soluble mediators; involvement of active phase reactants; and activation of the complement and other reactive humoral cascade systems. Although the manifestation of many exercise-induced immune and related changes has been reported and confirmed repeatedly, the underlying mechanisms triggering and modulating the elicited immune responses are, at best, poorly understood. The exaggerated and sometimes uncontrollable inflammatory response seen in septic and trauma patients results in morbidity and mortality, but strenuous exercise normally elicits only a sub-clinical inflammatory response that facilitates the repairing process for site-specific tissue damage. Regardless of the inciting event, for example trauma, infection or exercise, a remarkably similar sequence of inflammatory reactions can be reproduced in an affected host that is given an appropriate triggering signal. Therefore, physical exercise and training represent an acceptable, and possibly a good model for the study of subclinical inflammatory responses in humans.

Introduction

Strenuous muscular work is known to induce cellular and humoral changes in the body, and some of these changes are readily measurable in the blood (Shephard, 1997; Shephard and Shek 1996). To a large extent, the detectable changes in the circulation appear to be very similar, if not identical to those seen in traumatic injuries and sepsis (Northoff et al. 1995). One or more of the five cardinal signs of inflammation, namely redness, swelling, heat, pain and loss of function, are evident during and after strenuous physical activity (Smith 1991). Exercise of sufficient intensity and duration is invariably accompanied by activation of different components of a classical acute inflammatory response (Shephard and Shek 1996). Although the instigators of the inflammatory response in sepsis have been shown to be bacterial products such as endotoxins (Heumann and Glauser 1994), the nature of the inciting agents responsible for triggering the inflammatory cascade in exercise remains elusive.

The patient population with inflammatory disorders is a potential source of subjects for research, but there have been only limited experimental controlled studies of the inflammatory response in humans, for obvious ethical reasons. Experimental endotoxemia, for example, has been exploited to

investigate not only the cellular and humoral changes associated with induced inflammatory reactions, but also the potential efficacy of therapeutic interventions (Santos and Wilmore 1996). In contrast to the use of toxins as an invasive way to trigger an inflammatory reaction, physical exercise offers a possibly good alternative model to study the inflammatory cascade in a non-invasive manner. This symposium has highlighted some of the features of the inflammatory response seen in trauma, sepsis and exercise, and it has presented evidence to substantiate the notion that exercise can be used as a model to study subclinical inflammatory responses in humans.

Basis of inflammation

Inflammation represents a series of reactions occurring in the body in response to tissue damage and infection (Gallin et al. 1988). The main features of the inflammatory response include vasodilation that increases blood flow to the affected site; increased vascular permeability to facilitate the diffusion of soluble mediators across the endothelial barrier; and cellular infiltration by inflammatory cells at the site of injury (Austin and Wood 1993). The inflammatory focus may contain blood-derived cells such as neutrophils, basophils, eosinophils, and lymphocytes, or tissue-derived cells such as tissue macrophages, mast cells and tissue fibroblasts (Chrousos 1995). Circulating cells, e.g., lymphocytes and neutrophils, roll on stimulated endothelium before attaching to sites of inflammation or vascular injury (Ahmed and Christou 1996). The cells anchor themselves to the endothelial wall by the expression of adhesion molecules, notably selectins and integrins, in a process called margination; this facilitates the migration of cells into the nearby tissue site (Mackay and Imhof 1993; Frenette and Wagner 1996). During inflammation, numerous cellular and humoral events are orchestrated by the body in an attempt to restore homeostais, including leukocytosis and leukocyte activation; infiltration by granulocytes and monocytes/macrophages; activation of the acute phase response; release of inflammatory mediators; production of free radicals; activation of the complement system; and activation of the coagulation and fibibrinolytic cascades (Camus et al. 1993).

Inflammatory mediators: IL-1, IL-6 and TNF

Soluble mediators are intimately involved in mediating and modulating the inflammatory process (Dinarello 1991; Heumann and Glauser 1994). These mediators include substances derived by activation of a number of proteolytic cascade systems in the blood plasma, such as the complement, coagulation, kinin, and fibrinolysis systems. Products of arachiodonic acid metabolism, such as leukotrienes and prostaglandins are also mediators of inflammation. Among a long list of mediators, a small group of cytokines, particularly IL-1, Il-6 and TNF, are known to be the key immune mediators in an inflammatory response (Dinarello 1991; Heumann and Glauser 1994). Cytokines of this group are proinflammatory factors, playing an active role in the early phase of the inflammatory sequence (Pyne 1994). They synergise with one another to activate the acute phase

response after tissue injury and microbial insults; the result is the synthesis and release of acute phase proteins which include, for example, some complement components, a1-antitrypsin and haptoglobin. Each of the cytokines mediate pleiotropic effects during the effector phase of inflammation (Bone, 1996).

IL-1 is primarily synthesized by cells of the monocyte/macrophage lineage and its production can be triggered by infection, injury and other immunological stimuli (Dinarello and Wolff 1993). IL-1 can exert multifunctional effects by interacting with numerous cell types (di Giovine and Duff 1990). One of the key functions of IL-1 is in activation of T cells, which results in IL-2 production and the expression of IL-2 receptors on the cell surface (Dinarello 1988). During an inflammatory response, IL-1 induces neutrophils to migrate from the bone marrow to a tissue site via the blood stream by serving as a chemoattractant (Austin and Wood 1993). IL-1 stimulates hepatocytes to produce acute phase reactants and induces endothelial cells to upregulate their expression of adhesion molecules to increase leukocyte adherence (Dinarello 1988). IL-1 also induces collagenase production in fibroblasts and it promotes cartilage and calcium resorption in bones (Dayer et al. 1986; Dinarello 1988). IL-1 acts on macrophages/monocytes during inflammation, inducing its own synthesis as well as the production of TNF and IL-6 (Dinarello 1988; 1991).

IL-6 is another multifunctional cytokine not produced constitutively by normal cells. The secretion of this cytokine is triggered by infection and tissue injury (Hoch et al. 1993). The pleiotropic effects of IL-6 include the promotion of B cell development; activation of T cells; and induction of the acute phase response (Van Snick 1990). IL-6 possesses a number of growth factor activities, promoting cell growth and differentiation (Austin and Wood 1993). IL-6 contributes to the body's defence after infection by inducing fever and stimulating the release of adrenocorticotropic hormone (Van Snick 1990; Chrousos 1995). IL-6 is also known to synergize with IL-1 in inducing the cytotoxic T lymphocyte (CTL) response (Austin and Wood 1993). Overall, IL-6 plays an important role in mediating the inflammatory and immune responses initiated by infection and injury.

Tumor necrosis factors (TNF-α and TNF-β) possess a multitude of biological activities in the acute phase response to infection and injury, and in modulating cell growth and differentiation (Beutler and Cerami 1989; Vassalli 1992). TNF-a, produced primarily by activated macrophages, is also known as cachectin, because it was found to cause wasting or cachexia in animals with chronic infections or malignant growths (Beutler and Cerami 1989). The production of TNF-α is induced by trauma, tissue damage, and infectious stimuli (Damas et al. 1989; Hoch et al. 1993). TNF-α triggers the synthesis and release of other mediators such as proteases, prostaglandins, and free radicals (Bachwich et al. 1986). This pleiotropic cytokine also promotes the release of inflammatory

mediators such as IL-1 and PGE2 by macrophages, and it increases the adhesion of lymphocytes and neutrophils to endothelial cells (Pober and Cotran 1990). TNF-β is produced by activated lymphocytes, but not by macrophages. The function of TNF-β is mainly lymphotoxic and its production can be induced by injury; this cytokine is an important mediator of the delayed type hypersensitivity inflammatory response (Austin and Wood 1993).

Sepsis, endotoxemia, and proinflammatory cytokines

In Gram-negative sepsis, the presence of toxins stimulates the production of a variety of cytokines and inflammatory mediators with specific and interdependent tissue effects (Heumann and Glauser 1994; Damas et al. 1992; 1997), but there is no doubt that the endotoxin or lipopolysaccharide (LPS) derived from the bacterial cell wall is the primary instigator. Endotoxin is perhaps one of the most potent inducers of an inflammatory response. Among the proinflammatory mediators, TNF, IL-1 and IL-6 appear in the early part of the inflammatory response and their appearance at the site of inflammation follows a specific temporal sequence, with TNF-a appearing first, IL-1 second, and IL-6 last (Chrousos 1995).

Elevations in circulating TNF and IL-6 levels occur within 1.5 to 3 h following the induction of endotoxemia in humans (Santos and Wilmore 1996). IL-1, however, remained undetectable in plasma following endotoxin administration, prompting the authors to hypothesize that IL-1 is "inactive" in human endotoxemia. In contrast to this hypothesis, IL-1 has been implicated as a crucial mediator of septic shock, because it can cause tachycardia and hypotension as well as synergize with TNF to cause tissue damage and death (Okusawa et al. 1988; Everaerdt et al. 1989). The important role of IL-1 in endotoxin-induced inflammatory reactions is further evidenced by the demonstration that administration of a recombinant interleukin-1 receptor antagonist (IL-1ra) effectively reduces the lethality of endotoxin-induced shock in rabbits (Hannum et al. 1990; Ohlsson et al. 1990). Therefore, any failure to detect circulating IL-1 in endotoxemia is insufficient in itself to preclude the contribution of this cytokine to the pathogenesis of septic shock.

In contrast to the generally more elusive detection of circulating IL-1 in endotoxemia and sepsis, the other two proinflammatory cytokines, TNF and IL-6, are readily detectable in experimental and clinical sepsis (Ayala et al. 1992; Ertel et al. 1991; Martich et al. 1991; Wagge et al. 1989; Damas et al. 1989). In a model of chronic sepsis, an increase in TNF-a release was also found to be accompanied by elevated transcriptional activity of the gene encoding the proinflammatory cytokine (Hadjiminas et al. 1994). In normal humans receiving an intravenous injection of endotoxin, peak TNF immunoreactivity occurred at 1.5 h, followed by IL-6 at 2 -3 h, with no detectable IL-1β in any treated subject (Martich et al. 1991). In a human model of experimental endotoxemia, Ottaway et

al. (1997) also observed that peak levels of circulating TNF were reached about 30 min earlier than those for IL-6. In the same study, a concomitant increase in circulating cortisol levels was evident and its appearance was postulated to exert an inhibitory effect on TNF-α and IL-6 synthesis in the inflammatory response, presumably at the transcriptional level (Zanker et al. 1990). In patients with meningococcal septic shock, the detection of IL-1 was inconsistent, but TNF-α and IL-6 were detectable in all patients examined (Waage et al. 1989). Again, peak circulating TNF-a release occurred shortly before IL-6; both cytokines have been implicated as important mediators in septic shock and an apparent relationship was found between levels of either cytokine and fatal outcome (Cerami and Beutler 1988; Waage et al. 1987; Waage et al. 1989). Based on an analysis of plasma proinflammatory cytokine concentrations in critically ill patients admitted to an intensive care unit, Friedland et al. (1996) also concluded that bioactive TNF in plasma was an independent factor indicating a poor prognosis. Their findings are consistent with the concept that TNF is involved in the early phase of the inflammatory response and that IL-6 is secreted later and for a longer period. In contrast to the value of TNF in predicting outcome for critically ill patients, TNF-α and IL-6 were optimal markers for defining patients with septic shock, in terms of their sensitivity, specificity, and predictive value, despite the fact that both cytokines reached the highest concentrations in patients with acute septic shock (de Werra et al. 1997).

Exercise and proinflammatory cytokines

Cannon and Kluger (1983) first detected a proinflammatory cytokine with pyrogenic activity in plasma obtained from human subjects who had exercised for 1 h at 60% VO2max. The active moiety of the exercise-induced mediator was found to be the same as IL-1 (Cannon et al. 1986). In contrast to detectable IL-1 levels after exercise under laboratory conditions, no change in plasma IL-1 concentration was found in a field study where military cadets underwent a 7-day physically demanding ranger training course (Boyum et al. 1996). It is not clear whether the lack of detectable changes in IL-1 under field conditions may reflect confounding variables such as energy deficiency, sleep deprivation and psychological stress associated with the training course. More recently, Bury et al. (1995) reported that the exercise-induced elevation in plasma IL-1 level was correlated with exercise intensity; the greater the exercise intensity, the greater the increase in plasma IL-1 level. Plasma IL-1 activity is maximal a few hours after exercise and returns to basal levels by 24 h (Cannon et al., 1986). Following use of eccentric exercise as a noninfectious inflammatory stimulus, IL-1β was found in skeletal muscle tissue for up to 5 days (Cannon et al. 1989). Although IL-1β can augment protease production by fibroblasts and chondrocytes, facilitating the removal of damage tissue in a catabolic capacity (Dayer et al. 1986), it also helps to promote smooth muscle cell growth and augment collagen production in an anabolic manner (Krane et al. 1985; Libby et al. 1988).

Plasma levels of two other proinflammatory cytokines, TNF-a and IL-6, are elevated after strenuous exertion. Circulating TNF-α was increased by approximately 43, 53 and 64% in subjects who completed a 5-km, 2.5-h and 2.8-4.7 h run, respectively (Table 1) (Espersen et al. 1990; Dufaux and Order 1989; Camus et al. 1997). In two studies in which subjects ran for 10 and 20 km, no increases in circulating TNF-α were observed, but urinary TNF-α was found in subjects participating in the 20-km race (Camus et al. 1994; Sprenger et al. 1992). Smith et al. (1992) found no significant changes in plasma TNF-α or IL-6 levels in subjects after completing 1 h of cycling at 60% of maximal oxygen uptake. Following a 250-km competitive cycling race by well-conditioned athletes, plasma TNF-α level had increased by 130% (Gannon et al. 1997), although their absolute values appeared low compared to those reported in other studies (Table 1). Strenuous exercise induces a dramatic increase in plasma IL-6 concentration. A 79-fold increase in circulating IL-6 was found in a run that required 2.8-4.7 h to finish (Camus et al. 1997), and the completion of a 250-km competitive cyling race in about 6.5 h boosted the IL-6 level 45 fold (Table 1) (Gannon et al. 1997). By comparison, a 10-km run only triggered an 8-fold increase in plasma IL-6 concentration (Camus et al. 1994). Thus, exercise-induced increases in circulating TNF-α and IL-6 levels bear some sort of a relationship to the degree of physical exertion.

Difference in TNF-α and IL-6 concentrations induced by exercise versus septic complications

TNF-α and IL-6 are becoming increasingly used in exercise and clinical studies, where the monitoring of proinflammatory cytokines is required. Under normal resting conditions, the baseline levels of both cytokines is relatively low, but their circulating concentrations can surge quite readily in strenuous exertion or among critically ill patients with traumatic or septic complications. A consistent pattern of TNF-α and IL-6 response can be reproduced in experimental endotoxemia (Santos and Wilmore 1996; Ottaway et al. 1997). Although the magnitude of the cytokine response varies between studies, the increase in circulating TNF-α and IL-6 concentrations is generally much larger in experimental and clinical endotoxemia than during strenuous physical activity (Tables 1 and 2). In patients who develop septic shock, the magnitude of TNF-a and IL-6 levels can become exceedingly high, in the ng/mL range (Table 2) (Waage et al., 1989; Damas et al. 1997). IL-6 is related to mortality, and an IL-6 concentration above 1 ng/mL is associated with a significantly increased risk of death (Damas et al. 1992; 1997). Recognizing that performing 3-6 h of continuous exercise in a marathon or cycling race induces a circulating level of IL-6 to no greater than 100 pg/mL (Table 1), it is extremely unlikely that exercise-induced IL-6 elevation per se could be a life-threatening factor.

Instigators of the inflammatory response

Inflammation is a manifestation of the response of the body to tissue damage and infection. The instigator of the inflammatory response is obvious in traumatic injury (where tissue damage occurs) and in sepsis (where microbial products such as endotoxins are released) (Heumann and Glauser 1994; Northoff et al. 1995). The trigger of inflammation and proinflammatory mediators in exercise is less obvious. At least two possible mechanisms have been proposed to account for activation of the inflammatory response in exercise, namely muscle injury and bacterial translocation from the gut.

Muscle injury

Damage to skeletal muscle fibers occurs after strenuous physical exercise (Fridén et al. 1983). Ultrastructural and morphological changes have been described at the site of muscle injury, where infiltration by polymorphonuclear leukocytes is a common feature (Fielding et al. 1993). A significant accumulation of intramuscular neutrophils was observed in biopsies obtained 45 min after downhill running, and neutrophil infiltration persisted for up to 5 days after exercise. Specific immunohistochemical staining also revealed the accumulation of IL-1β at the ssite of muscle damage. The post-exercise neutrophil influx has been suggested as serving to clear tissue damage in preparation for repair and cell growth (Camus et al. 1993). Thus, invading inflammatory cells appear to be the consequence of the damage, in an attempt to remove cellular debris, rather than its cause.

Leukocytosis is a hallmark of the exercise-induced cellular changes (McCarthy and Dale 1988) and leukocyte activation is an important part of the inflammatory response (Shephard and Shek 1996). The majority of the increase in circulating leukocyte count is due to a granulocytosis, which persists into recovery (Shek et al. 1995; Shephard and Shek 1996). Other contributors are a rise in lymphocyte and moncocyte counts. The rapid and persistent leukocytosis is believed to be due to a demargination of cells from the non-circulating cell pool (McCarthy and Dale 1988). The demarginated leukocytes in prolonged exercise such as distance running are mainly polymorphonuclear (PMN) leukocytes. Since exercise of high intensity and long duration is likely to increase muscle damage, it is conceivable that the continuous release of damaged cell materials could serve as a potent signal, triggering PMN recirculation and activation. Tidball (1995) proposed that a substance released from damaged muscle initiates the inflammatory response; the activation of resident macrophages or fibroblasts by a "wound hormone" may provide the necessary signals to attract additional inflammatory cells to the injured site. Basic fibroblast growth factor, platelet derived growth factor, IL-1 and TNF-α are involved in mediating muscle inflammation and repair. These growth-promoting and repair mediators are known to be released

by activated resident macrophages. IL-1, for example, can serve a range of functions in the early stages of inflammation by acting as a chemoattractant and recruiting additional inflammatory cells. TNF-α also exerts multiple effects in the inflammatory response, such as inducing IL-1 synthesis (Bachwich et al. 1986) and skeletal muscle proteolysis (Goodman 1991). The biological effects of TNF-α and IL-1 can be further amplified by interferon-g (Northoff et al. 1995). In summary, exercise and muscle damage increase IL-1 and TNF-α production and the elevated proinflammatory cytokines contribute to the inflammatory or regererative phases of muscle reponse to injury.

Bacterial translocation from the gut

During strenuous exercise, the redirection of blood flow to the active muscles and skin causes transient hypoperfusion of the gut, sometimes resulting in splanchnic ischemia (Moses, 1993; Kenney and Ho, 1995). A reduced blood supply to the intestine could compromise the barrier function of the gut wall, resulting in the translocation or spillage of bacterial products such as endotoxins to the circulation (Marshall and Nathens 1996). Endotoxins can activate the complement cascade via the alternative pathway, resulting in the production of C3a and C5a, both of which are powerful complement cleavage products, capable of causing vasodilation and increased vascular permeability (Kuby 1994). As an anaphylatoxin, C5a can trigger the degranulation of granulocytes and mast cells, further exacerbating the inflammatory process. Endotoxins are mitogenic and they stimulate lymphocyte proliferation. By activating the Hageman factor (factor XII), endotoxins can also trigger the coagulation and fibrinolytic cascades (Heumann and Glauser 1994). Thus, the release of endotoxins from the gut can significantly amplify the inflammatory response.

Systemic endotoxemia has been documented in athletes after prolonged and highly demanding physical exertion (Bosenberg et al. 1988; Brock-Utne et al. 1988; Moore et al. 1995; Camus et al. 1997). An ultradistance triathlon competition increased the mean LPS concentration among competing athletes (Bosenberg et al. 1988). Endotoxemia greater than 100 pg/mL was also observed in exhausted ultramarathoners, who had participated in a 89.4-km race (Brock-Utne et al. 1988) and most of the runners who developed endotoxemia displayed gastrointestinal symptoms. Moore et al. (1995) examined the relationship between endotoxemia and mild post-exertional illness in cyclists who had completed a 160-km road race. Although endotoxemia was evident in some cyclists, no causal relationship to post-competition illness was found. More recently, Camus et al. (1997) reported that some marathoners who had participated in a 2.8-4.7 h race, developed moderate, transient endotoxemia, but again no correlation between endotoxemia and the magnitude of the inflammatory response was observed. Thus, although endotoxemia has been repeatedly

demonstrated after strenuous exercise, presumably because of endotoxin translocation from the gut, the pathophysiological role of circulating endotoxin in exercise-induced inflammatory response remains obscure.

Despite the detection of endotoxemia in athletes who have performed exhaustive endurance exercise, direct evidence of a causal relationship between post-exercise illness and inflammatory response is lacking. Endotoxin or LPS binds to CD14 on the surface of monocytes/macrophages; these cells then become activated, releasing a whole host of inflammatory products, including the proinflammatory mediators IL-1, IL-6 and TNF-a (Heumann and Glauser 1994). The minimal in-vivo dose of endotoxin required to activate phagocytic cells is not known, but it is quite possible that sufficient LPS molecules could have leaked from the gut to trigger the initial events of inflammation, in the absence of detectable endotoxemia. Therefore, failure to detect endotoxemia does not necessarily indicate a lack of involvement of endotoxin in triggering the inflammatory events. There is a need for more sensitive assays to measure LPS that has escaped from the gut and is bound to target cells, in order to confirm or refute the hypothesis of bacterial translocation as a possible mechanism instigating the inflammatory response in exercise.

Conclusions

Strenuous muscular work can trigger the initiation of an inflammatory cascade, characterized by a series of cellular and humoral changes qualitatively similar to, but quantitatively different from those seen in trauma and sepsis. The underlying mechanisms responsible for the exercise-induced cytokine and cellular inflammatory response are far from clearly understood. A huge collection of studies in trauma, infection, and exercise has identified, at the very least, tissue injury and bacterial products, as the dominant instigators of the ensuing inflammation. The inflammatory response is initiated and propagated by highly complex, but intertwined cellular and humoral mediators. Two sets of cellular and humoral mediators, composed of a *cellular triplet* of PMN, macrophage/monocyte, and lymphocyte and a *cytokine triplet* of IL-1, IL-6 and TNF-α, in conjuction with the acute phase response, play a major role in mediating and regulating inflammation.

The process of inflammation is an innate response of the body to restore "law and order" and re-establish homeostasis, in the face of physical injury and infectious insult. Trauma, infection, and septic complications can drive the inflammatory cascade to such an excessive extent that it passes out of control, with detrimental or even fatal consequences. Shock resulting from sepsis, for example, is associated with uncontrolled, excessive production of proinflammatory mediators (Heumann and Glauser 1994). On the other side of the coin, exercise-induced inflammatory

response is normally subclinical in nature and it is *ordered* and *controlled.* In this regard, the concerted response is for promoting repair and regrowth, making the response a beneficial one.

Regardless of the inciting event, whether it be trauma, infection or exercise, and given an appropriate triggering signal, a remarkably similar sequence of inflammatory reactions can be reproduced in the affected host. Although it is debatable whether the triggering mechanisms are the same in each case, the inflammatory cascade itself has undeniable resemblance. Therefore, physical exercise and training may represent an acceptable, and possibly a good model for the study of subclinical inflammatory responses in humans.

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Appendix 3
Publications

Peer-reviewed papers, abstracts, and presentations completed to date with support of contract.

Peer-Reviewed Research Papers (1996-2000)

1. Shephard, R.J. & Shek, P.N. (1996). Exercise and CD4+/CD8+ cell counts: Influence of various contributing factors in health and HIV infection. Ex. Immunol. Rev. 2: 65-83.

2. Cross, M.C., Radomski, M.W., VanHelder, W.P., Rhind, S.G. and Shephard, R.J. (1996). Endurance exercise with and without a thermal clamp: effects on leukocytes and leukocyte subsets. J. Appl. Physiol. 81: 822-829.

3. Brenner, I.K.M., Severs, Y.D., Shek, P.N. and Shephard, R.J. (1996). Impact of heat exposure and moderate, intermittent exercise on cytolytic cells. Eur. J. Appl. Physiol. 74: 162-171.

4. Mertens, D.J., Rhind, S., Berkhoff, F., Dugmore, D., Shek, P.N. & Shephard, R.J. (1996). Nutritional, immunological and psychological responses to a 7250 km run. J. Sports Med. Phys. Fitness 36: 132-138.

5. Rhind, S.G., Shek, P.N., Shinkai, S. & Shephard, R.J. (1996). Effects of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin 2 (IL-2) production, and IL-2 receptor expression. Eur. J. Appl. Physiol. 74: 348-360.

6. Severs, T., Brenner, I., Shek, P.N. & Shephard, R.J. (1996). Effects of heat and intermittent exercise on leukocyte and subpopulation counts. Eur. J. Appl. Physiol. 74: 234-245.

7. Shephard, R.J. and Shek, P.N. (1996). Impact of physical activity and sport on the immune system. Reviews on Environmental Health 11: 133-147.

8. Shephard, R.J. & Shek, P.N. (1996). Physical activity and immune changes: a potential model of subclinical inflammation and sepsis. Crit. Rev. Phys. Rehabil. Med. 8: 153-181.

9. Shephard, R.J. & Shek, P.N. (1997). Autoimmune disorders, physical activity and training, with particular reference to rheumatoid arthritis. Exerc. Immunol. Rev. 3: 53-67.

10. Shinkai, S., Konishi, M. & Shephard, R.J. (1997). Aging, exercise, training and the immune system. Exerc. Immunol. Rev. 3: 68-95.

11. Gannon, G.A., Rhind, S., Suzui, M., Shek, P.N. & Shephard, R.J. (1997). Circulating levels of peripheral blood leucocytes and cytokines following competitive cycling. Can. J. Appl. Physiol. 22: 133-147.

12. Shephard, R.J. & Shek, P.N. (1997). Interactions between sleep, other body rhythms, immune responses, and exercise. Can. J. Appl. Physiol. 22: 95-116.

13. Brenner, I.K.M., Thomas, S., and Shephard, R.J. (1997). Spectral analysis of heart rate variability during heat exposure and repeated exercise. Eur. J. Appl. Physiol. 76: 145-156.

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15. Shephard, R.J. (1997). What is the optimal type of physical activity to enhance health? Br. J. Sports Med. 31: 277-284.

16. Brenner, I., Shek, P.N., Zamecnik, J., and Shephard, R.J. (1998). Stress hormones and the immunological responses to heat and exercise. Int. J. Sports Medicine 19: 130-143.

17. Shephard, R.J. and Futcher, R. (1997). Physical activity and cancer: How may protection be maximized? Crit. Rev. Oncogen. 8: 219-272.

18. Shore, Susan and Shephard, R.J. (1998). Immune responses to exercise and training: A comparison of chidlren and young adults. Ped. Exerc. Sci. 10: 210-216.

19. Shephard, R.J. & Shek, P.N. (1998). Acute and chronic over-exertion: Do depressed immune responses provide useful markers? Int. J. Sports Med. 19: 159-171.

20. Shephard, R.J. & Shek, P.N. (1998). Immunological hazards from nutritional imbalance in athletes. Exerc. Immunol. Rev. 4: 22-48.

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31. Shephard, R.J. & Shek, P.N. (1998). Cold exposure and immune function. Can. J. Physiol. Pharm. 76: 828-836.

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33. Shore, S., Shinkai, S., Rhind, S. & Shephard, R.J. (1999). Immune responses to training: How critical is training volume? J. Sports Med. Phys. Fitness 39: 1-11.

34. Shephard, R.J. (1999). Advances in Exercise Immunology, by. L. Mackinnon. Book Review. Can. J. Appl. Physiol. 24: 291-292.

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36. Rhind, S.G., Gannon, G.A., Suzui, M., Shephard, R.J., Shek, P.N. (1999). Indomethacin inhibits circulating PGE2 and reverses postexercise suppression of natural killer cell activity. Am. J. Physiol. 276: R1496-1505

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38. Rhind, S. G., G. A. Gannon, P. N. Shek, I. K. M. Brenner, Y. Severs, J. Zamecnik, V. M. Natale, R. M., A. Buguet, R. J. Shephard and M.W. Radomski (1999). Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. J. Appl. Physiol., 87: 1178-1185.

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40. Brenner, I.K.M., Natale, V.M., Vasiliou, P., Moldoveanu, A., Shek, P.N. & Shephard, R.J. (1999). Impact of three different types of exercise on components of the inflammatory response. Eur. J. Appl. Physiol. 80: 452-460.

41. Shephard, R.J., Castellani, J.W., & Shek, P.N. (1998). Immune deficits induced by strenuous exertion under adverse environmental conditions: Manifestations and countermeasures. Crit. Rev. Immunol. 18: 545-568.

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53. Shephard RJ, Gannon G, Hay JB, Shek PN. (2000). Adhesion Molecule Expression in Acute and Chronic Exercise, Crit. Rev. . In press.

54. Gannon GA, Rhind SG, Shek PN, Shephard RJ. (2000). Differential Cell Adhesion Molecule Expression and Lymphocyte Mobilization during Prolonged Aerobic Exercise. Eur J Appl Physiol. Submitted for publication.

55. Seabrook TJ, Ristevski B, Rhind SG, Shek PN, Zamecnik J, Shephard RJ, Hay J. (2000). Epinephrine causes a reduction in lymph node cell output in sheep. Can J Physiol Pharmacol. Submitted for publicxation.

56. Gannon GA, Rhind SG, Shek PN, Shephard RJ (2000). Mobilization of LFA-1a$^{+/-}$ and L-selectin+/- naive and memory,
$CD4^{+}$ and $CD8^{hi}$ T cell subsets during aerobic exercise in the heat. Paper in preparation.

57. Rhind SG, Castellani JW, Brenner IKM, Shephard RJ, Zamecnik J, Montain S, Young AJ, Shek PN. Intracellular and serum cytokine profiles following exhausting exercise and cold exposure. Paper submitted for publication.

Abstracts (1996-2000)

1. Rhind, S.G., Gannon, G.A., Shek, P.N. and Shephard, R.J. (1996). Enhanced release of natural killer cell stimulating factor (interleukin-12) during prolonged exercise. Physiologist 39: A-46.

2. Gannon, G.A., Rhind, S.G., Shek, P.N. and Shephard, R.J. (1996). Exercise-induced changes in the expression and surface density of cellular adhesion molecules on circulating CD56+ lymphocytes. Physiologist 39: A-46.

3. Cross, M.C., Radomski, M.W., Shephard, R.J., VanHelder, W., & Rhind, S.G. (1997). Leukocyte and hormonal responses to sustained aerobic exercise with and without a core temperature clamp. Int. J. Sports Med. 18: S102.

4. Gannon, G.A., Rhind, S.G., Suzui, M., Shek, P.N., & Shephard, R.J. (1997). Changes in selected cellular and soluble mediators of immunity following a 250-km competitive road-cycling race. Int. J. Sports Med. 18: S108.

5. Rhind, S.G., Gannon, G.A., Suzui, M., Shek, P.N., & Shephard, R.J. (1997). Determination of natural killer cell activity by flow cytometry: applications in studies of exercise immunology. Int. J. Sports Med. 18: S113.

6. Shinkai, S., Komura, T., Asai, H., Konishi, M. & Shephard, R.J. (1997). Physical activity and immune senescence in elderly men. Int. J. Sports Med. 18: S103.

7. Shephard, R.J., Kavanagh, T. & Mertens, D.J. (1997). Predicting physiological and psychological response to aerobic training in stable chronic heart failure. Med. Sci. Sports Exerc. 29: S270.

8. Gannon, G.A., Rhind, S.G., Suzui, M., Shek, P.N. & Shephard, R.J. (1997). Exercise-enhanced natural killer cell cytotoxic capacity of peripheral blood is not affected by the opioid antagonist naltrexone. Med. Sci. Sports Exerc. 29: S297.

9. Rhind, S., Shore, S., Shinkai, S. & Shephard, R.J. (1997). Immune responses to exercise and training: Is there a training volume effect? Can. J. Appl. Physiol. 22: 50P.

10. Shephard, R.J. & Shore, S. (1997). Immune responses to exercise: A comparison of children and young adults. Can. J. Appl. Physiol. 22: 54P.

11. Shephard, R.J. & Shore, S. (1997). Immune responses to exercise in children treated for cancer. Can. J. Appl. Physiol. 22: 54P.

12. Brenner, I.K.M., Natale, V.M., Vasiliou, P., Moldoveanu, A.I., Shek, P.N., and Shephard, R.J. (1998). Impact of different types of exercise on NK cells, cytokines and creatine kinase. Med. Sci. Sports Exerc. 30: S19 (Abstr.).

13. Suzui, M., Nagao, F., Takeda, K., Yagita, H. Okumura, K., Rhind, S.G., Gannon, G.A., Shek, P.N., and Shephard, R.J. (1998). Is ventilatory threshold a key to open the window of natural killer cell cytotoxicity? Med. Sci. Sports Exerc. 30: S19 (Abstr.).

14. Rhind, S.G., Gannon, G.A., Shek, P.N., Suzui, M., and Shephard, R.J. (1998). Effects of 2 h of exercise and in vivo indomethacin on circulating PGE2 levels and NK cell activity. Med. Sci. Sports Exerc. 30: S20 (Abstr.).

15. Gannon, G.A., Rhind, S.G., Shek, P.N. and Shephard, R.J. (1998).Is the differential lympocyte subset mobilization during exercise linked to subset expression of lymphocyte function-associated antigen (LFA-1)? Med. Sci. Sports Exerc. 30: S21 (Abstr.).

16. Shephard, R.J., Shore, S. Immune responses to exercise and training in children treated with chemotherapy. Int. J. Sports Med. 19 (Suppl. 3) S213 (Abstract).

17. Gannon, G., Rhind, S.G., Shek, P.N., Shephard, R.J. (1998). The majority of CD4+, but not CD8hi, T cells mobilized to the peripheral blood during exercise express a CD45RO+ memory phenotype. Int. J. Sports Med. 19 (Suppl. 3) S213 (Abstract).

18. Rhind, S.G., Shore, S., Shinkai, S., Shephard, R.J. (1998). Immune responses to exercise and training: Is there a training volume effect? Int. J. Sports Med. 19 (Suppl. 3) S213 (Abstract).

19. Shephard, R.J., Shore, S. (1998). Immune responses to exercise and training: A comparison of children and young adults. Int. J. Sports Med. 19 (Suppl. 3) S213 (Abstract).

20. Suzui, M., Nagao, F., Takeda, K., Yagita, H., Okumura, K., Rhind, S.G., Gannon, G.A., Shek, P.N., Shephard, R.J. (1999). Do temporary anaerobic efforts open the window of natural killer cell cytotoxicity? Med. Sci. Sports Exerc. 31: S61 (abstr.).

21. Gannon, G.A., Rhind, S.G., Shek, P.N., Shephard, R.J. (1999). Opioid antagonism during prolonged moderate intensity exercise: Effects on circulating leucocyte and lymphocyte subset mobilization. Med. Sci. Sports Exerc. 31: S62 (abstr.).

22. Rhind, S.G., Gannon, G.A., Brenner, I.K.M., Natale, V.M., Shek, P.N., Shephard, R.J. (1999). Contribution of hyperthermia to exercise-induced lymphocyte subset redistribution. Med. Sci. Sports Exerc. 31: S62 (abstr.).

23. Rhind, S.G., Gannon, G.A., Brenner, I.K.M., Natale, V.M., Shek, P.N., Shephard, R.J. (1999). Contribution of hyperthermia to exercise-induced lymphocyte subset redistribution. Med. Sci. Sports Exerc. 31: S62 (abstr.).

24. Suzui, M., Nagao, F., Takeda, K., Yagita, H., Okumura, K., Shek, P.N. & Shephard, R.J. (2000). Changes in natural killer cell cytotoxicity and adhesion molecules during incremental exercise. Med. Sci. Sports Exerc. 32: S50 (Abstr.).

25. Rhind, S.G., Shek, P.N., Brenner, I.K., Castellani, J.W., Montain, S.J., Young, A.J. & Shephard, R.J. (2000). Intracellular and serum cytokine profiles after 8 days of exhaustive exercise and cold exposure. FASEB Journal 14, A618 (Abstr.).

26. Gannon, G.A., Rhind, S.G., Shek, P.N., Zamecnik, Y., Shephard, R.J. (2000). Inhibition of exercise- induced cytokine release by thermal clamping. FASEB Journal 14: A619 (abstr.).

Presentations (1996-2000)

1. Shephard, R.J. Invited lecture: Physical activity and immune functioning.International Conference on Healthy Aging, Activity and Sports, Heidelberg, August 1996.

2. Shephard, R.J. Invited lecture: Exercise, aging, and immune resistance to infections and neoplasms. *Ibid.*

3. Shephard, R.J. Invited lecture: Physical activity in health promotion. Tokyo Medical College, WHO Collaborating Centre for Health Promotion through Research and Training in Sports Medicine, September, 1996.

4. Shephard, R.J. Invited key-note lecture. Assumptions inherent in biological testing. North American Society for Adapted Physical Activity, Banff, September, 1996.

5. Rhind, S., Gannon, G.A., Shek, P.N. & Shephard, R.J. Enhanced release of natural killer cell stimulating factor (interleukin-12) during prolonged exercise. Presentation to joint APS/CSEP meeting, Vancouver, October, 1996.

6. Gannon, G.A., Rhind, S., Shek, P.N. & Shephard, R.J. Exercise-induced changes in the expression and surface density of cellular adhesion molecules on circulating CD56+ lymphocytes. Presentation to the joint APS/CSEP meeting, Vancouver, October, 1996.

7. Shephard, R.J. Exercise and the Immune System. Invited lecture, St. Paul's Hospital and School of Rehabilitation Sciences, University of British Columbia Symposium on Exercise Physiology and Active Rehabilitation: Integrating Research and Clinical Practice, 1996.

8. Shephard, R.J. Invited lecture, University of New Brunswick, Fredericton, January 1997. Exercise and the Immune System, 1997.

9. Gannon, G.A, ,S.G. Rhind, M. Suzui, P.N. Shek, & R.J. Shephard Exercise-enhanced natural killer-cell cytotoxic capacity of peripheral blood is not enhanced by the opioid antagonist naltrexone. American College of Sports Medicine, Denver, May 1997.

10. Shephard, R.J. Physical Activity in an aging population: implications for health. Keynote lecture, German Society of Sport Sciences, Symposium on Training im Altersport. Bonn, May 1997.

11. Shephard, R.J. Immune changes induced by exercise in an adverse environment. Invited paper presented at International Symposium on "Immune responses to inflammation and trauma: A physical training model", Toronto, July 1997.

12. Shephard, R.J. Invited keynote lecture, American Academy of Sports Physicians, Toronto, July 1997. Exercise and the Immune System.

13. Rhind, S., Shore, S., Shinkai, S. & Shephard, R.J. Immune response to exercise and training. Is there a training volume effect? Canadian Society of Exercise Physiology, Toronto, October, 1997.

14. Shephard, R.J., and Shore, S. Immune responses to exercise: A comparison of children and young adults. Canadian Scoiety of Exercise Physiology, October, 1997.

15. Shephard, R.J. & Shore, S. Immune responses in children treated for cancer. Canadian Society of Exercise Physiology, October, 1997.

16. Shephard, R.J. & Shore, S. Immune responses to exercise and training in children treated with chemotherapy. International Symposium on Exercise and Immunology, Paderborn, November, 1997.

17. Gannnon, G.A., Rhind, S., Shek, P.N. & Shephard, R.J. The majority of CD4+ but not CD8+ T cells mobilized to peripheral blood during exercise express a CD45RO' memory phenotype. *Ibid*, November, 1997.

18. Rhind, S., Shore, S., Shinkai, S. & Shephard, R.J. Immune responses to exercise and training: is there a training volume effect? *Ibid*, November, 1997.

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Shephard, R.J.

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14. ABSTRACT

(U) Sepsis is a major cause of morbidity and mortality in combat casualties. A major problem in developing successful treatment has been the lack of appropriate human experimental models. Conclusions from animal experimentation have been suspect because of inter-species differences in the nature and time course of inflammatory reactions from those encountered in human surgery. Prolonged and strenuous physical activity can in itself cause substantial clinical injury, potentially causing an excessive inflammatory reaction and immunosuppression which mirrors that seen following surgical trauma This has opened up prospects of developing a technique that would permit controlled studies of adverse immune reactions to trauma The objectives of this contract were thus to develop an exercise model that maximized cellular and humoral immune changes, and to use this model to explore patterns of secretion of pro- and anti-inflammatory cytokines and hormones during the stress of heavy exercise

A laboratory comparison of brief, near maximal effort, sustained aerobic exercise, and a circuit of resistance exercise found that, contrary to expectations, sustained aerobic exercise above the anaerobic threshold yielded the reactions most typical of trauma. However, no mode of laboratory exercise induced a sustained and prolonged inflammatory response. In contrast, six hours of competitive cycling induced large increases in the secretion of pro-inflammatory cytokines. The depression of immune function induced by many forms of laboratory exercise seems too brief to have great practical importance for health. If such changes were induced several times per week, as in a sustained operation, there might be a cumulative adverse effect on immuno-surveillance and health experience. However, field studies of a basic training course at CFB Meaford found no adverse effects on health

Given that laboratory exercise has only moderate effects on immune function, trials of counter-measures for sepsis will require either tests during more intensive training, such as the US Ranger training, or an exacerbation of the immediate inflammatory response by exposure to the combined stressors likely in combat, for example, extremes of heat and/or cold, and a negative energy balance. There seems some increase in resting immune function as individuals become trained, and this can partially offset the adverse effects of the inflammatory reaction.

Future research on exercise models of sepsis should concentrate upon the development of more marked immune changes through combinations of stressors, the testing of additional pro- and anti-inflammatory cytokine responses as concentrations of these substances fall within detection limits, and an examination of the protective effects of other anti-oxidants and nutritional supplements.

Les infections sont l'une des principales causes de morbidité et de mortalité chez les blessés de guerre. Jusqu'ici, il a été difficile de mettre au point un traitement efficace, notamment parce qu'il n'existait pas de modèle expérimental humain adéquat. Les constatations découlant de l'expérimentation animale sont jugées suspectes parce que la nature et l'évolution des réactions inflammatoires diffèrent d'une espèce à l'autre et de ce qu'on observe en chirurgie humaine. L'activité physique prolongée et intensive peut entraîner des dommages cliniques assez importants et déclencher une réaction inflammatoire excessive ainsi qu'un effet d'immunosuppression correspondant à ce qu'on voit après une chirurgie. Avec l'observation de ce phénomène est apparue la possibilité de mettre une technique au point pour étudier en conditions contrôlées les réactions immunitaires nuisibles dues à des traumatismes. Les objectifs du projet décrit ici étaient donc de mettre au point un modèle d'exercice permettant de changer au maximum les fonctions immunitaires cellulaires et humorales, et d'utiliser ce modèle pour étudier la sécrétion des hormones et des cytokines pro-inflammatoires et anti-inflammatoires durant le stress d'un exercice intense

En comparant au laboratoire les effets d'un bref exercice, exécuté en aérobie constante près du niveau d'effort maximal, à ceux d'une série d'exercices de résistance, on a constaté que, contrairement à toute attente, l'exercice en aérobie constante au-dessus du seuil d'anaérobiose entraîne des réactions très caractéristiques d'un traumatisme Toutefois, aucune forme d'exercice en laboratoire n'a déclenché de réaction inflammatoire constante et durable. Par contre, six heures de vélo de compétition a fait augmenter dans une mesure importante la sécrétion de cytokines pro-inflammatoires. Il semble que la dépression de la fonction immunitaire que de nombreuses formes d'exercice de laboratoire ont entraînée ne dure pas assez longtemps pour avoir une véritable incidence sur la santé Si ce genre de changement survenait plusieurs fois par semaine, comme il arrive durant une opération militaire de longue durée, il pourrait donner lieu à un effet nuisible cumulatif pour l'immuno-surveillance et l'expérience de santé Toutefois, lorsque l'entraînement de base a été examiné dans des études réalisées sur le terrain à la base des Forces canadiennes de Meaford, rien ne dénotait d'effet nuisible pour la santé.

Comme l'exercice en laboratoire n'a que des effets modérés sur la fonction immunitaire, pour faire l'essai de

moyens de lutte contre les infections, il faudra des exercices plus intensifs, comme ceux de l'entraînement des US Ranger, ou une exacerbation de la réaction inflammatoire immédiate par exposition à l'ensemble des facteurs de stress susceptibles d'intervenir au combat, comme, par exemple, un froid et/ou une chaleur extrêmes et un bilan énergétique négatif. Par ailleurs, la fonction immunitaire de repos semble s'accroître à mesure que l'entraînement progresse, ce qui peut contrebalancer en partie les effets nuisibles de la réaction inflammatoire.

Il conviendrait dorénavant que la recherche sur les modèles d'exercice pour l'étude des infections soit axée sur l'obtention de changements immunitaires plus marqués par la combinaison de facteurs de stress, l'évaluation d'autres réactions pro-inflammatoires et anti-inflammatoires faisant intervenir des cytokines lorsque leur concentration est comprise entre les limites de détection et l'étude des effets protecteurs d'autres anti-oxydants et suppléments nutritifs.

15. KEYWORDS, DESCRIPTORS or IDENTIFIERS

(U) stress; exertion; physical activity; inflammation; immune; lymphocytes; cytokines; cytotoxicity

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